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Vesicle-Surfactant Interactions: Effects of Added Surfactants on the Gel to Liquid-crystal Transition for Two Vesicular Systems

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Interactions of both cationic and anionic surfactants with vesicles formed by dimethyldioctadecylammonium bromide (DOAB) and by sodium didodecylphosphate (DDP) have been probed using differential scanning microcalorimetry. The scans show that the surfactants are incorporated into the vesicle bilayers. The change in the melting temperature, T_m, characterising the gel to liquid-crystal transition depends on whether the charges on the head groups of surfactant ion and vesicular ion have either similar or opposite signs.

Synthetic vesicular systems[1] are interesting from many standpoints, not the least being their structural relationship with the phospholipid component of biologically important membranes.[2] In addition, synthetic vesicular systems offer interesting opportunities to probe interactions involving membrane systems. Interactions of surfactants with biopolymers[3] are important in many different contexts particularly because they are the key component of detergents, but also because of their many important applications in biochemical research. Almost all of these applications rely upon the ability of the surfactant to solubilise many hydrophobic components.[4] For example, high concentrations of sodium *n*-dodecyl sulfate (SDS) will unfold and solubilise globular proteins, disrupt biological membranes, and will release and stabilise many membrane-bound proteins. The object of this study was to probe the nature of the interaction between added surfactant and model bilayer systems in an attempt to understand the process involved in the initial binding to, then disruption of, the bilayer structure.

Such interactions are complex and often difficult to quantify, we have been exploring the use of microcalorimetry in this regard. In this present study we report on the nature of the interaction between vesicle systems and added surfactants. In aqueous solutions, both dioctadecyldimethylammonium bromide[5] and sodium didodecyl phosphate[6] (DDP) form vesicles. We have explored the interaction of these vesicular systems with surfactants and, although the interactions are undoubtedly complex,[7] we have shown in this study that patterns emerge that have a pleasing self-consistency.

The investigation centred on the gel to liquid-crystal transition as recorded using differential scanning microcalorimetry. Previously[5] we showed that, in the case of dimethyldioctadecylammonium bromide (DOAB), the key transition occurs at 44.8 ± 0.1 °C. The dependence of the isobaric heat capacity, C_p, on temperature could also be understood in terms of transitions within local domains (patches). For DOAB [aq; 2×10^{-3} (mol monomer) dm^{-3}] we estimated that *ca.* 130 DOAB monomers form such a patch, even though each vesicle contains many thousands of monomers. The associated enthalpy of melting was estimated as 8.90 kcal (mol monomer)$^{-1}$. For sodium DDP[6] [aq; 2×10^{-3} (mol monomer) dm^{-3}] the transition centred on 35.1 ± 0.1 °C with a patch number of *ca.* 250 and an enthalpy of melting equal to 2.57 kcal (mol monomer)$^{-1}$. Striking evidence of vesicle-surfactant interaction is reported based on the scans recorded by DSC for solutions of both DOAB and DDP when SDS, sodium dodecyl phosphate (SDP) or hexadecyltrimethylammonium bromide (CTAB from the trivial name) are added. For comparison, we report the effects of added hexanol on the DSC scans for DOAB vesicles.

Experimental

Materials

The preparation of DDP (solute) has been described.[8] DOAB (Aldrich) was used as purchased.[1] We have shown that the protocol for preparation of the aqueous solutions is vitally important.[5,6,9,10] In particular, we have shown[5,6,9,10] that the ethanol injection method is suspect, the traces obtained using DSC being irreproducible not only during repeat scans but also using freshly prepared solutions. A similar state of affairs exists[10] when scans are compared for vesicle solutions prepared using the ethanol injection method to which has been added SDS, CTAB or hexanol.

To prepare the DOAB solutions, a weighed amount of the solid was dissolved in water and the solution heated to 50 °C and held at this temperature for 10 min while being stirred. The solution was allowed to cool to and remain for 1 h at room temperature. A weighed amount of either CTAB or SDS was added with stirring. The solution was placed in the sample cell of the calorimeter, thermostatted at 15 °C.

The DDP solutions were prepared in a similar fashion except that the solution was initially heated to above 55 °C and held at that temperature for half an hour with stirring.

Calorimetry

A MicroCal differential scanning microcalorimeter was operated in the manner previously described.[11] The output from the DSC produced plots showing the dependence of differential heat capacity on temperature. We described previously[1] how this dependence can be transposed into a dependence of molar heat capacity on temperature describing a transition which involves a group (patch) of monomers in the bilayer. We found that tbe transitions near 45 °C for DOAB [aq; 2×10^{-3} (mol monomer) dm^{-3}] and 35 °C for DDP [aq; 2×10^{-3} (mol monomer) dm^{-3}] could be accounted for in terms of simple two-state equilibria.[11] This was not always

the case when CTAB or SDS were added (see below). In certain cases, the ORIGIN software[11] could be used to fit the dependences of heat capacity on temperature to more than one independent two-state equilibria, *e.g.* X ↔ Y and W ↔ Z.

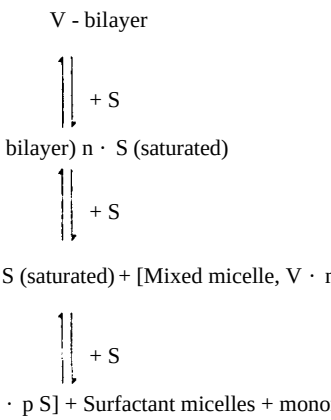
Mixed Solutions

The protocols described above were successful in producing aqueous solutions containing vesicles. However, there were some failures which are significant. Three examples make the point. Attempts were made to prepare aqueous solutions containing (i) DOAB (4.2×10^{-3} mol dm^{-3}) + equimolar DDP, (ii) DOAB (2×10^{-3} mol dm^{-3}) + DDP (10^{-3} mol dm^{-3}) and (iii) DOAB (2×10^{-3} mol dm^{-3}) + DDP (5.0×10^{-4} mol dm^{-3}). After adding the appropriate weighed amounts of DDP and DOAB to water, the solutions were heated to 70 °C and stirred for 10 min. On cooling, white precipitates were produced by the three solutions.

Results and Discussion

The formation of a stable bilayer structure is critically dependent on monomers with two alkyl chains that pack favourably in a lamellar form with limited head-group interactions. For monomers containing only a single alkyl chain the hydrophobic-hydrophilic balance is altered in such a way that head-group repulsion destabilises the bilayer structure in favour of the micellar structure. The addition of surfactant to a model bilayer system is expected to lead through a series of equilibria ultimately to disruption of the bilayer. The bilayer will progressively take up surfactant until it becomes saturated, at which point further addition of surfactant will lead to disruption of the bilayer and concomitant formation of mixed micelles (see Scheme 1).[3] Previous studies have monitored this process of disruption of bilayer structures by monitoring the release of a fluorescent or radioactive solute encapsulated within unilamellar vesicles.[12] This present study has focused on the early steps in this process of disruption.

The effects of surfactant on the properties of vesicle solutions have been investigated through DSC studies on an extensive series of solutions containing different concentrations of either DOAB or DDP to which had been added varying concentrations of the surfactants CTAB, SDS or SDP, and for comparison hexanol. The general phenomena through the various concentrations of vesicle solutions were the same and, therefore, we comment here in detail on a single vesicle concentration of 2×10^{-3} mol dm^{-3} of either DOAB or DDP. However, it is important to note that in these studies the temperatures of the solutions are changed, whereas most other studies (*e.g.* ref. 12) examine surfactant-vesicle interactions at a single temperature. In this context, it is also important to recall that the critical micellar concentrations (c.m.c.s) of surfactants may depend on temperature. As points of reference, the c.m.c.s for surfactants[13] used in the present study are as follows. For CTAB the c.m.c. increases with increase in temperature; 0.955×10^{-3} mol kg^{-1} at 298 K to 2.61×10^{-3} mol kg^{-1} at 368 K. For SDS, the c.m.c. shows a small dependence on temperature being in the region of 9×10^{-3} mol dm^{-3} from 5 to 55 °C. In the DSC experiments reported here, the prepared concentrations are close to the c.m.c.s although we do not mean that the surfactants are present as either monomers or micelles in the presence of either DOAB or DDP.



Scheme 1

A typical set of scans is reported in Fig. 1 which shows the effect of added CTAB on the DSC traces for DOAB (aq; 2×10^{-3} mol dm^{-3}). In the absence of CTAB, two extrema are recorded at 36 and 44.8 °C. The transition at 36 °C was linked[6] to the melting of patches perturbed by vesicle-vesicle interaction/aggregation. The transition at 44.8 °C was identified[6] as the main gel to liquid-crystal transition.

Two features of the plots in Fig. 1 are clear-cut. Addition of CTAB at the lowest concentration removes the feature at 36 °C indicating a marked decrease in vesicle-vesicle interactions. In these terms, added CTAB cations and bromide anions present in the aqueous phase between the vesicles insulates the vesicles from one another within the organised solution. Turning attention to the high-temperature feature, the temperature T_m decreases with an increase in CTAB concentration. For each solution, the pattern formed by the first scan differed from those recorded on subsequent scans over the range 15 to 70 °C. Clearly, the addition of surfactant to solutions of pre-formed vesicles gives systems that are not initially at equilibrium. This may be associated with the ability of the surfactant to penetrate the gel state but once the system is scanned through the gel to liquid-crystal transition, the system comes to equilibrium and all subsequent re-scans are fully reproducible. Implicit in this observation is the fact there must be a very slow step; furthermore, it is possible that the system present after the first scan is, in fact, a metastable state.

One further complexity emerged from the shape of the dependence of dC_p on temperature which could not be accounted for in terms of a single two-state equilibrium. Instead analysis showed that two independent processes are involved, consistent with two types of patches. We interpret

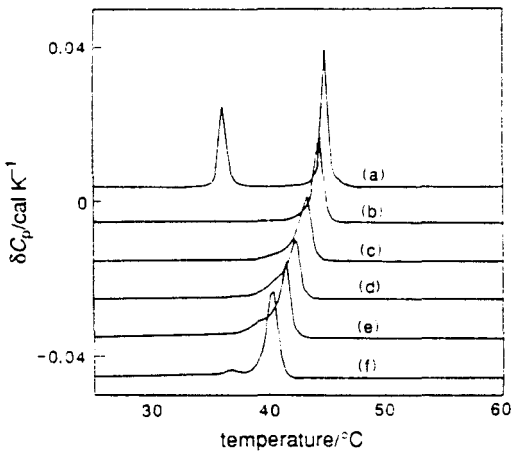


Fig. 1. Dependence of differential isobaric heat capacities on temperature for DOAB (aq; 2×10^{-3} mol dm^{-3}) containing different concentrations of CTAB; (a) 0, (b) 5×10^{-4}, (c) 10^{-3}, (d) 1.5×10^{-3}, (e) 2×10^{-3} and (f) 2.5×10^{-3} mol dm^{-3}. [For clarity the curves have been displaced on the heat capacity axis; (b)-(f) report second scans (see text).]

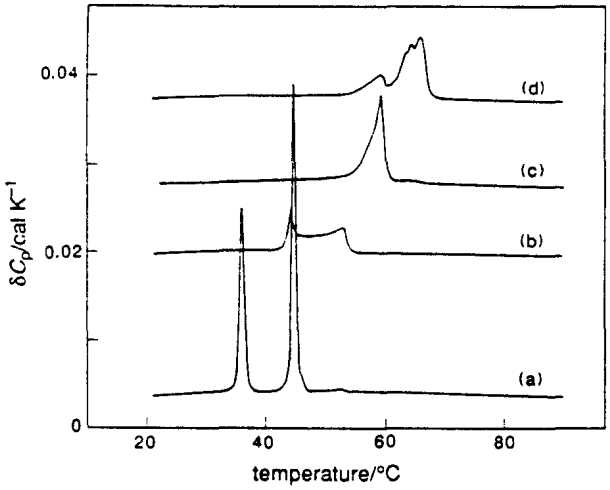


Fig. 2 Dependence on temperature of differential heat capacity for DOAB (aq; 2×10^{-3} mol dm^{-3}) containing different concentrations of SDS; (a) 0, (b) 5×10^{-4}, (c) 10^{-3} and (d) 1.5×10^{-3} mol dm^{-3} (For clarity the curves have been displaced on the heat capacity axis)

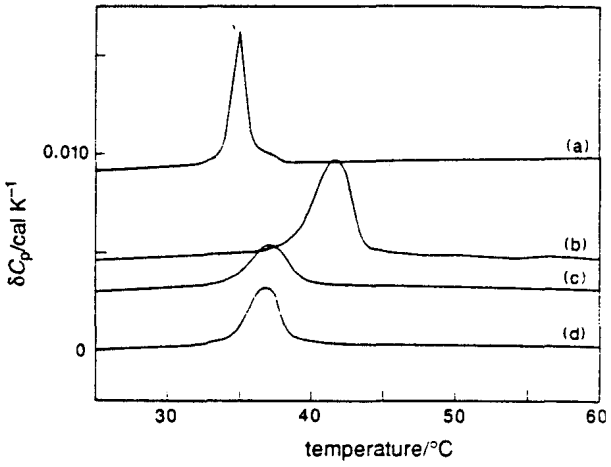


Fig. 4 Dependence on temperature of differential heat capacity for DDP (aq; 2×10^{-3} mol dm^{-3}) containing (a) no additive and 1.5×10^{-3} mol dm^{-3}, (b) CTAB, (c) SDS and (d) SDP

this to mean that some patches are richer in CTAB than others. If the CTAB cations insert into the bilayers with the alkyl chains adjacent to dodecyl chains, the mismatch in length must lower the thermal stability. A similar trend was noted[14] in the DSC scans for vesicles formed from di-alkylphosphates (sodium) where the two alkyl chains differ in length. We conclude that the lower temperature component contributing to the envelope near 40 °C characterises domains which are CTAB rich.

The pattern in Fig. 1 reflects the similarities between DOAB and CTAB monomers; common bromide anions and an 'onium type head group to the alkyl chains. A more dramatic effect might be expected when SDS was added to DOAB; Fig. 2 records the effect of increasing concentrations of added SDS. The contrast between the effects of added SDS and CTAB is highlighted in Fig. 3 where we also include the scans when SDP is added.

Just as for CTAB, addition of the two new surfactants obliterates the low-temperature transitions. However, the scans resulting from addition of SDS are more complicated than in the case of CTAB. Despite the complexity, the overall effect is to raise the temperature associated with the transition. In other words, the gel phase is stabilised but the eventual melting process is less clearly defined. Presumably the patches within the DOAB vesicle contain a range of local concentrations of SDS. We presume that the C_{12}-chains pack into the DOAB (C_{18}-chains) and may lead to some disruption of the structure but the negatively charged head groups of SDS in the surface of the vesicle counter the head-group repulsion between $\rangle N^+Me_2$ groups. The latter raises the thermal stability of the gel state. The very broad scan produced by added SDP is a consequence of the poor packing of the di-negative phosphate head group within the surface leading to poorly defined melting.

The importance of the head group in determining the melting characteristics is borne out by the comparison drawn in Fig. 4. Based on the effect of added CTAB on T_m for DOAB we had predicted that, by adding SDS to DDP, T_m would decrease because both head groups have the same charge sign. This turned out not to be the case. Instead, both cationic and anionic surfactant ions raised the T_m. In the case of SDS (Fig. 4), T_m increases from 35 to 38 °C although the shift is small. In fact, the overall scan could be accounted for in terms of three independent transitions (Fig. 5).

The patterns which emerge for the DOAB and DDP systems when surfactants such as SDS and CTAB are added point to incorporation of the surfactants within the vesicles.

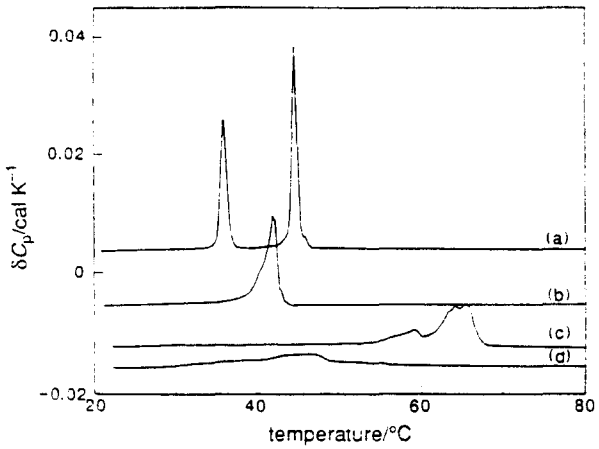


Fig. 3 Dependence on temperature of the differential heat capacity for DOAB (aq; 2×10^{-3} mol dm^{-3}) containing (a) no additive and 1.5×10^{-3} mol dm^{-3}, (b) CTAB, (c) SDS and (d) SDP (The scans have been displaced for clarity on the heat capacity axis)

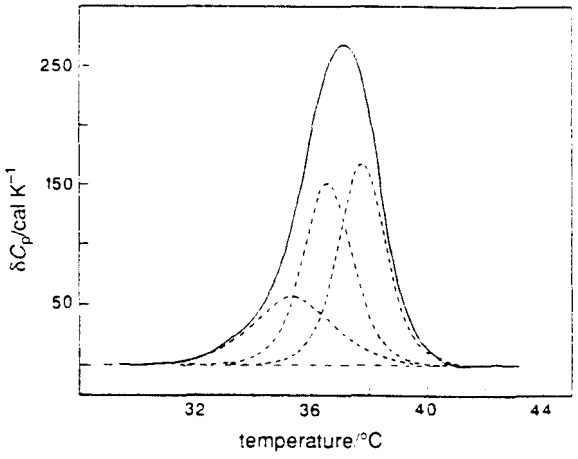


Fig. 5 Analysis of the dependence of isobaric heat capacity on temperature for DDP (aq; 2×10^{-3} mol dm^{-3}) containing SDS (1.5×10^{-3} mol dm^{-3}; recorded scan (——) and calculated components (- - -)

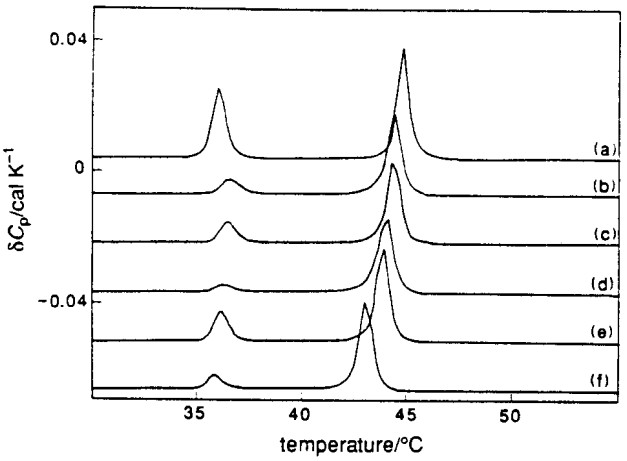


Fig. 6 Dependence on temperature of differential heat capacity for DOAB (aq; 2×10^{-3} mol dm^{-3}) containing added hexanol; (a) none (b) 5×10^{-4}, (c) 10^{-3}, (d) 1.5×10^{-3}, (e) 2×10^{-3} and (f) 2.5×10^{-3} mol dm^{-3} (The curves have been displaced on the heat capacity axis for clarity)

We suggest that such dramatic changes in the scans reported here could not occur if the surfactants were located in the aqueous phases between the vesicles. The dramatic changes at such low surfactant concentrations point to profound influences on the thermal stabilities. However these influences are not necessarily a consequence simply of the alkyl chains in the surfactants. These chains allow the surfactants to become incorporated into the bilayer structure but the major impact on the thermal stability is attributable to the charged head group. This conclusion is supported by the set of scans produced for solutions containing increasing concentrations of hexanol (Fig. 6). With an increase in hexanol concentration the intensity of the low-temperature extremum falls but the temperature at the maximum hardly changes. In contrast, T_m for the main extremum falls gradually. Although not directly comparable, the latter trend is reminiscent of the 'depression freezing point' by an added solute. However, we attribute the trend to a decreasing stability of the gel state following incorporation of hexanol into the vesicles. The impact of the polar OH head group of the hexanol appears to have little impact.

Although the data reported here confirm the complexity of surfactant-vesicle interactions, two important conclusions are drawn. First, DSC offers an extremely sensitive probe of these interactions. Secondly, the surfactants influence the gel-liquid phase transition by incorporation into the vesicle. Thirdly, the alkyl chains in the added surfactant destabilise the gel state but this trend can be countered by a charged polar head group.

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